

Comments on "Short Exposures to 60 Hz Magnetic Fields Do Not Alter MYC Expression in HL60 or Daudi Cells" by Saffer and Thurston (*Radiat. Res.* **144**, 18–25, 1995)

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Saffer and Thurston recently concluded that 60-Hz magnetic fields do not alter *MYC* mRNA in HL60 cells (1). They said that their study was "well-controlled" and a "significant effort," and that their "inescapable" conclusion "refutes" the work of others, particularly that of Goodman *et al.* (2, 3). In my view, despite the authors' definitive tone and prepublication publicity (4, 5), the report contained significant errors. (1) The study was not negative, as the authors claimed. (2) Even if it were negative, it would not refute the work of Goodman *et al.* (3) Even if it refuted Goodman *et al.*, it would not show that 60-Hz magnetic fields do not affect *MYC*. (4) Contrary to the authors' claims, the study did not and cannot have a meaningful impact on assessment of the potential link

between electromagnetic fields (EMFs) and cancer. The report had other flaws in experimental design, statistics, procedure and interpretation that are sufficient by themselves to invalidate the authors' conclusion.

1. The variance at 5.7 μ T differed significantly ($F = 4.95$, $P < 0.05$) from that in the sham-exposed group (lines 1 and 2 in Table I). Thus their data showed that the magnetic field altered *MYC* expression, contrary to the authors' conclusion. The values of n were too low for the remaining data in the table to provide reasonable assurance or reliability of any conclusion (the work in the Henderson laboratory performed by the authors had $n = 2$, and most of their other experiments had $n \leq 5$).

2. Saffer and Thurston normalized their data using β -2 microglobulin mRNA, but in the work that they intended to replicate (2, 3), Goodman *et al.* reported that *all* expressed genes were up-regulated by the field. Their seminal publications also made it clear that the applied fields caused an increase in *total* mRNA (6, 7). Consequently, Saffer and Thurston used a wrong procedure because, if the field affected expression of both the test and control genes, the authors would falsely conclude that there had been no effect of the field. They justified their normalization procedure by labeling it an internal control for loading the gels, but any gain in loading accuracy would have been trivial because even large pipetting errors ($\pm 5\%$) would have been relatively insignificant and could not have influenced the results unless they all occurred in the same direction for the E or C.

Saffer and Thurston are in a dilemma regarding β -2 microglobulin. They maintained that inclusion of an internal reference was important, and their data (Table I) were normalized using β -2 microglobulin, which they claimed was unresponsive to the magnetic field. How do they know? They could not rely on Goodman for such knowledge because that would have been grossly inconsistent with the purpose of their experiment, which was to replicate her data. For the sake of consistency they should have employed an internal standard to assess β -2 microglobulin's unresponsiveness, because it was the lack of such a standard that prompted their criticism of Goodman and led to the authors' claim that their study was an "improvement." Yet it seems clear that the authors did not use a non- β -2 microglobulin internal reference.

What little evidence of β -2 microglobulin mRNA that was presented (Fig. 3) suggested that the transcript was affected by the field.

3. Saffer and Thurston asserted conclusions not sustained by their data when they claimed that fields "do not alter *MYC* expression," "do not damage the genome," "do not alter the ability of the cells to repair damage." None of these statements is or can be correct because the empirical evidence offered in each case was simply the absence of a difference between the treated and control cells. Banal as it may sound, the failure to reject the null hypothesis does not prove the negative.

4. Saffer and Thurston said that "the plausibility of an EMF-cancer link has rested, in large part, on the previous reports of increased expression of an oncogene," but this opinion is unsupported by evidence. Goodman's work was plausible because of the many reports of EMF-induced effects on increased cell growth (8-11). Based on such reports it is reasonable to expect altered protein and mRNA in stimulated cultures, *MYC* included; such data, however, are not evidence that EMFs are linked to cancer. Since the occurrence of a generalized increase in protein and mRNA levels does not indicate that EMFs cause cancer, it follows logically that the absence of increased levels is not evidence that EMFs do not cause cancer.

5. The authors employed energized double-wound coils to provide sham exposure. If such coils *must* be used (not the case here), great care is needed to ensure that the windings remain balanced throughout the exposure period, because small temperature differences can cause a current imbalance, resulting in the application of a field to the control flasks. Saffer and Thurston provided no justification for the use of double-wound coils and no documentation that the coils remained balanced during all exposures. Investigators at the authors' institution (PNL) have a history of advocating complex exposure systems for EMF studies that ultimately fail, causing artifacts; such was the case with their exposure systems for mice (12, 13), rats (14) and pigs (15-17). There is therefore considerable precedent for suspecting the reliability of excessively complex exposure systems designed at PNL.

Unjustified complexity was present throughout the study by Saffer and Thurston, and the reader is entitled to ask why these distracters were employed. For example, consider the authors' use of annular culture vessels. The issue that the authors addressed in their study (replication of the work of Goodman *et al.*) involved the *existence* of an effect, not the feature of the applied field that produced it. It would have been logical to use flasks similar to those used by Goodman *et al.* (2, 3). Then, if their results were replicated, the question regarding the causal role of the induced electric field (the biophysical factor pertinent to the shape of the culture vessel) could have been addressed. If, on the other hand, effects were not observed, the negative result could not be attributed to a difference in culture vessels. Thus the authors introduced an irrelevant consideration into the experiment.

6. The authors reported that TPA produced changes in *MYC* expression that were said to be similar to those of others (18) who used a higher concentration of TPA (5 nM, compared with Saffer and Thurston's 3 nM). The similarity of the results indicated that their system was saturated, and yet it yielded an effect of less than a factor of 2. This suggests that the authors' assay could detect only powerful perturbations in cell regulation, thereby rendering their conclusion vulnerable to a Type II statistical error. If, for example, the effect produced by the magnetic field was one-fifth that of TPA, Saffer and Thurston probably would not have observed it. Thus it appears that their assay was relatively insensitive for determining field effects on *MYC* expression, especially considering that they evaluated the data (using an appropriate *n*) at only one time.

7. Saffer and Thurston repeatedly claimed to have measured "steady-state" *MYC* mRNA levels, but that claim is obviously untrue. The cells studied were added to culture flasks 16 h prior to a 20-min equilibration period that preceded the EMF exposure. Since the cells were growing rapidly, the notion of steady-state levels of *MYC* (or, for that matter, any mRNA) is meaningless because the cells were actively dividing and they were measured at only one time.

8. The magnitude and direction of the geomagnetic field differed between the authors' experimental and control chambers. Much research has suggested the biological significance of the geomagnetic field, and prior investigators have not employed different geomagnetic fields at the locations of the experimental and control chambers. Why these authors chose to do so, and then to ignore the implications in the Discussion, is a mystery.

9. The authors performed 18 experiments (Table I, line 1) at 7 different times, apparently with 2-3 independent exposed and control cultures (Fig. 3). But they did not explain how the results from different exposure periods were combined, how the E and C within exposure periods were combined, or what statistical test was performed to substantiate their conclusion of no effect. Within a given exposure period, for example, it would be possible to pair data for E and C in such a way as to minimize the departure of each ratio (and hence the average) from unity. Thus the statistical design of the study is obscure.

Whatever statistical procedures were in fact employed, the manner chosen for presentation of the results hid the effect of EMF exposure on variations in *MYC* mRNA which, as shown above, was affected by the field. The authors also failed to supply data in support of their factual claims that: (a) serum concentration and lot number had no effect; (b) neither the field nor TPA affected β -2 microglobulin mRNA; (c) the ddPCR results were entirely negative.

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